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EFFECTS OF *EVOLVULUS ALSINOIDES* LINN. IN INFLAMMATORY MARKERS IN PROSTATITIS INDUCED RAT MODEL

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ABSTRACT

The aim of this study was to investigate the anti-inflammatory effects of *Evolvulus alsinoides L.* in $AlCl_3$ induced prostatitis rat model. Prostatitis was induced in male Wistar rats (n=24) by treatment with $AlCl_3$ for 12 weeks. After the induction of prostatitis, the rats were randomly divided into one of four treatment groups namely Normal Control group (NC-group), prostatitis group ($AlCl_3$-group), *Evlovulus Alsinoides* leaves extract group (EALE group) and $AlCl_3$ + EALE group were used in the study. Inflammatory markers and The histo-pathological changes of the prostate were also examined. The EALE, showed effective anti-inflammatory activities in the prostate and the histological studies showed a considerable improvement in the prostatic histo-architecture of the groups fed EALE. It may be useful for the clinical treatment of nonbacterial prostatitis. Our findings suggest that EALE has a beneficial effect on the prevention and treatment of nonbacterial prostatitis.

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INTRODUCTION

Nonbacterial prostatitis is the most common urological diagnosis in men under 50 years of age and is the third most common urologic diagnosis in men over 50 years of age. It is marked by perineal pain radiating to the genital area, urinary symptoms, and ejaculatory disturbance, which have great impacts on the psychological and physiological status and quality of life of patients. An estimated 50% of all men experience prostatitis-like symptoms at some point during their lifetime[1]. The etiology and pathogenesis of nonbacterial prostatitis is unclear, so it is a difficult condition to treat. There is growing evidence that inflammation plays a significant role in

chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Thus, elevated levels of proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF-α), and IL-8 have been associated with diagnosis and symptom severity in patients with CP/CPPS.[2, 3] For this reason, anti-inflammatory medications have been used for the treatment of CP/CPPS.

Rat models of hormone-associated nonbacterial prostatitis can be useful for elucidating the mechanisms of the pathogenesis of nonbacterial prostatitis.[4] Wistar rats spontaneously develop nonbacterial prostatitis with advancing age, which makes them a good animal model for laboratory investigation of nonbacterial prostatitis.[5] Administration of exogenous estradiol (E2) increases the incidence and severity of prostatitis in adult Wistar rats.[5, 6] Naslund et al[5] reported that spontaneously-developed prostatitis and E2-induced prostatitis in Wistar rats had the same histologic findings. Other studies have demonstrated that spontaneous nonbacterial prostatitis in rats was histologically very similar to CP in humans.[7, 8]

Aluminium absorption and accumulation in humans can occur via the diet, drinking water, ingestion with fruit juices or citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of aluminium in healthy subjects[9]. Different forms of aluminium are environmental xenobiotics that induce free radical-mediated cytotoxicity and reproductive toxicity. High Aluminium contents in human testes, spermatozoa, seminal plasma, blood and urine, were associated with impaired sperm quality and viability[10,11,12] Alteration in the histology of testis and prostate[13,14] deterioration in spermatogenesis and sperm quality; enhancement of freeradicals and alterations in antioxidant enzymes; interruption in sex hormone secretion[15]are several of the aspects suggested that Aluminium exposure causes adverse impact on male reproduction. The aim of our study was to investigate the anti-inflammatory effects of *Evolvulus alsinoides linn* in the treatment of nonbacterial prostatitis in a rat model..

MATERIALS AND METHODS

Collection of plants:

The fully mature *Evalvulus alsinoides* Linn. whole plants were collected from marungulam, Thanjavur District, Tamil Nadu, India from a single herb. The collected leaves were identified and authenticated by a Botanist Dr. S.John Britto S.J, The Director, The Rapinat Herbarium and Center for molecular systematic, st.Joseph's College, Tiruchirappalli, Tamil Nadu. A Voucher specimen has been deposited at Tamil University Herbarium. The plants were cut into small pieces and shade dried and powdered finely then used for extraction.

Animals

Male albino rats of Wistar strain approximately weighing 190-200g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Preparation of plant extract:

The *Evolvulus Alsinoides* leaves were first washed well and dust was removed from the plant. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Preparation of Aluminum chloride ($AlCl_3$)

Two grams of aluminum chloride was dissolved in 100 ml distilled water to prepare a stock solution (20 mg/ml). The solution was prepared weekly and kept in a plane bottle at 4ºC. $AlCl_3$ was daily administrated to rats (0.1ml (2mg) /100gm) orally.

Experimental design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows.First group; was negative control administered 3 ml distilled water orally once daily. Second group; was positive control group ($AlCl_3$ group) administered aluminium chloride (20 mg/kg bw,), the LD 50 of $AlCl_3$ when administered orally to rats was reported to be (380 - 400 mg/kg bw (Krasovskii et al., 1979).Third group; was administered *Evolvulus alsinoides* leaves extract (EALE) (75 mg/kg bw) which dissolved in 3ml distilled water orally once daily according to Lekshmi and Reddy, (2011). Fourth group; was co administered with $AlCl_3$ and EALE in the same doses in 2nd and 3rd groups. Doses were given once daily via gavage for 70 consecutive days, for completion of the spermatogenic cycle and

maturation of sperms in epididymis (Sarkar et al., 2003). After 12 weeks of treatment, the prostatic proinflammatory cytokine (TNF-α, IL-6, and IL-8) levels and histological findings were noted.

Collection of blood and prostate tissues:

At the end of the experimental feeding period, the rats were fasted overnight and sacrificed under mild euthanasia with pentobarbital. Blood was collected by cardiac puncture into plain, heparinized and EDTA bottles, respectively for proinflammatory determinations. The blood in the plain bottles was allowed to clot and the serum separated at 3500 rpm for 15 min was used for determination of inflammatory marker. Prostate tissues were rapidly excised and fixed in 10% formyl saline.

Pro-inflammatory marker analyses

The N-acetyl-β-glucosaminidase activity was determine by the method of Walker and Pugh, (1960). The β –glucuronidas activity was determined earlier by the method of Fishman et al., (1948). NO concentration in the serum was measured by the method of Sastry*et al*., (2002). The cytokine concentration was measured every 5 minutes for 30 minutes, using a spectrophotometer at 450 nm with an immunoassay ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

Histological analysis

After the end of the experimental peroid, animals were sacrificed; organs such as Prostate, Testes, Liver, Kidney and Heart were removed and fixed for 4 days in 10% formaldehyde. After decalcification in 5% formic acid, processed for paraffin embedding tissue sections (7 µm thick) were stained with haematoxilin and eosin (Abudoleh *et al.*, 2011).

Statistical analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons (Harvey and Paige, 1998). The results were statistically analyzed by using SPSS (Statistical Packages for Social Studies) version 20 was used and $p< 0.05$ was considered to be significant.

RESULTS

1. **Pro-inflammatory marker analyses**

Inflammatory markers Nitric oxide, CRP, Homocystine, TNF- α, IL-6, and cortisol levels were found to be significantly higher in prostatic rats as compared with normal rats. Supplementation of EALE restored the levels of Nitric oxide, CRP, Homocystine, TNF- α, IL-6 and cortisol. The restoration with EALE treatment was statistically significant in Group IV. N-acetyl-β-glucosaminidaseb and β-glucuronidase activity were higher in Group II than Group I. N-acetyl-β-glucosaminidaseb and β-glucuronidase activity were restored in EALE treated group. Results shown in Table.1 and fig.1a &1b.

2**. Histological analysis:**

Sections of group I and III rats showed that the prostatic parenchyma was composed of packed acini of diferent sizes. Acini were lined with simple columnar cells with basal nuclei and a small number of epithelial papillary folds. The acini were separated by minimal fibromuscular stroma. Some acini contained homogenous acidophilic secretion.

Sections from the Group II aluminium chloride treated group showed larger epithelial cell layer and stromal space compared with controls. Desquamated cells were observed within the lumina of some acini. The acini showed many epithelial folds where the epithelial cells were arranged as multiple unorganized layers. Sections from the central region of ventral prostate of this group showed enormous dilatation of prostatic acini that were fled with secretion. Tinning and fattening of lining epithelium in some areas of the acini was clearly noted.

On examination of prostatic sections from Group IV aluminium chloride + *Evolvulus alsonoides* treated group, markedly improved glandular morphology was observed. The acini exhibited a reduction in the lining epithelium, reverting to the simple columnar form. Some epithelial cells exhibited small dark nuclei. Lumina of the acini appeared wide whereas the epithelial folds were diminished. The acini were separated with a reduced amount of stroma compared to the aluminium chloride treated group.

DISCUSSION

We determined that treatment with EALE decreased the expression of pro-inflammatory cytokines in a nonbacterial prostatitis rat model. We showed that EALE reduced TNF-α, IL-6, and IL-8 concentrations in the prostate tissue of nonbacterial

prostatitis rats. TNF-α secreted from macrophages is a trigger for the migration of activated macrophages and the production of several chemokines including IL-8 in inflammatory foci. Elevated IL-8 levels in the stroma of the prostate result in the accumulation of neutrophils and lymph cells, which suppress H_2O_2 production.[16] IL-8 also seems to be a key mediator in human benign prostatic hyperplasia (BPH). IL-8 concentrations in prostatic secretions from patients with BPH with inflammation are higher than in patients with BPH alone.[16] Penna et al[3] reported that IL-6 and IL-8 are significantly elevated in the semen and expressed in the prostatic secretions of men with CP/CPPS, and their levels are positively correlated with symptom scores. Taken together, these findings support the association of nonbacterial prostatitis with increased levels of proinflammatory cytokines.

Treatment with the multi-herbal medicine EALE led to decreased inflammation scores for the prostates from our nonbacterial prostatitis rat model. These effects are presumed to be the result of the anti-inflammatory effects of EALE. Prostatitis is not a known risk factor for prostate cancer but may increase the chance of its occurrence (Chang *et al*., 2012). The etiology of prostate inflammation is complex and not completely elucidated but involves age-related hormonal alterations, metabolic syndrome and inflammation (Thompson and Yang, 2000). Also, several studies have shown that other processes such as chronic inflammation and increased oxidative stress may play important roles in the development of prostatitis (Sciarra *et al*., 2008; Matsumoto *et al*., 2010).

We applied the 4-point inflammation grading system used by Bernoulli et al[17] to evaluate the severity of inflammation of the lateral prostate lobes. The severity of inflammation was assessed according to the aggressiveness of inflammation and by counting the number of inflamed acini from grade 0 to grade 3. In microscopic findings, reduced inflammation of the prostate and relieved degeneration of of the glandular epithelium were shown in the EALE treated rat group compared to those in the control group.

The ethanolic extract of *E. alsinoides has* different pharmacological activities such as gastro protective [18],antibacterial, antiulcer, immunodilatory cytoprotective,adaptogenic and antiamnesic, anxiolytic, analgesic and anti-inflammatory activity.Currently, it is prescribed as an herbal medicine for the treatment of chronic hepatitis and liver cirrhosis. Previous studies have shown that *evolvulus alsinoides* has cytoprotective effects against oxidative stress-induced hepatotoxicity and immunomodulatory effects.Oh et al[12] reported that *Evolvulus alsinoides* has anti-inflammatory activity in LPS-stimulated RAW 264.7 mouse macrophage cells.

We demonstrated that EALE improves AlCl3-induced nonbacterial prostatitis in rats by regulating pro-inflammatory cytokines but has anti-inflammatory effect. Therefore EALE is expected to have beneficial effects on the prevention and treatment of nonbacterial prostatitis in human patients.

CONCLUSIONS

Results from our current study suggest that the EALE, may have anti-inflammatory effects in a nonbacterial prostatitis rat model. Our finding that EALE treatment significantly suppressed pro-inflammatory cytokines (TNF-α, IL-6, IL-8) in the rat model of nonbacterial prostatitis suggests that this EALE may be useful for the clinical treatment of CP/CPPS in humans as well as contributing to the amelioration of prostate inflammation in BPH.

Table.1: Effect of *Evolvulus alsinoides* leaves on inflammatory markers in experimental rats

Parameters	Group I	Group II	Group III	Group IV
CRP (mg/dl)	2.80± 0.24	4.75±0.30 [a]	3.11±0.40	2.98±0.20
TNF-α (pg /ml)	10.02 ±0.72	15.80±1.04 [a]	10.40±0.74 [b]	9.44±0.52 [b]
IL-6 (pg /ml)	30.20 ±2.25	98.44±6.69 [a]	35.42±5.59 [b]	31.42± 3.21 [b]
Homocystine (µg/ml)	7.12± 0.53	13.87±0.921 [a]	8.32±0.80 [b]	7.80±0.69 [b]
N-acetyl-β-glucosaminidase (U /min/ml)	32.04 ±2.57	49.74±3.54 [a]	34.55.± 2.12 [b]	30.17± 2.46 [b]
β-glucuronidase (mU)	1.35± 0.08	3.12±0.29 [a]	2.16±0.19 [b]	.1.28±0.15 [b]
NO (µM/L)	25.41 ±1.87	54.72±3.41 [a]	30.74 ±2.25 [b]	28.14± 2.11 [b]

Values are expressed as mean ± SD for six rats in each group.

[a]Significantly different from group I ($p < 0.05$)

[b]Significantly different from group II ($p < 0.05$)

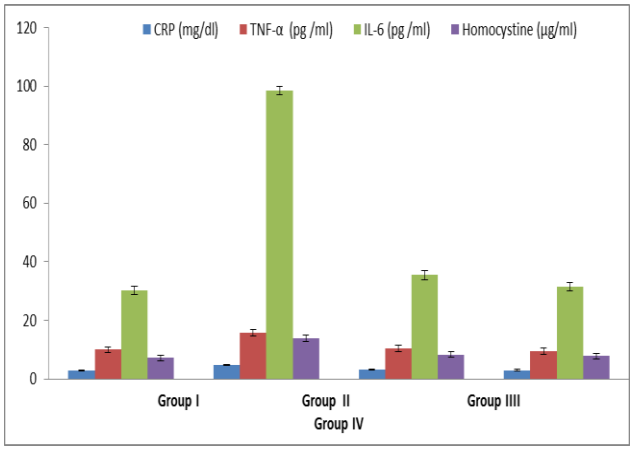

Fig.1.a: Effect of *Evolvulus alsinoides* leaves on inflammatory markers in experimental rats

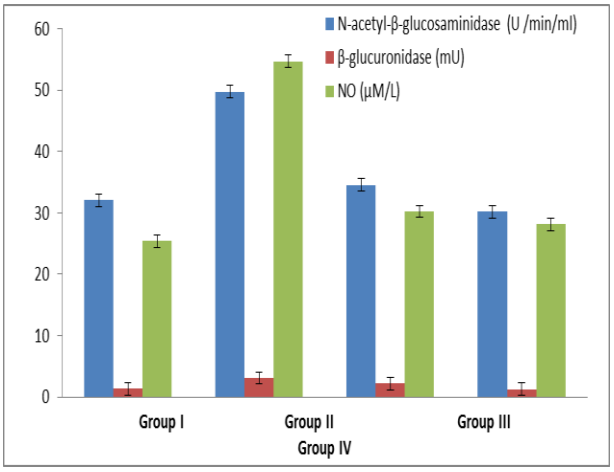

Fig.1.b: Effect of *Evolvulus alsinoides* leaves on inflammatory markers in experimental rats

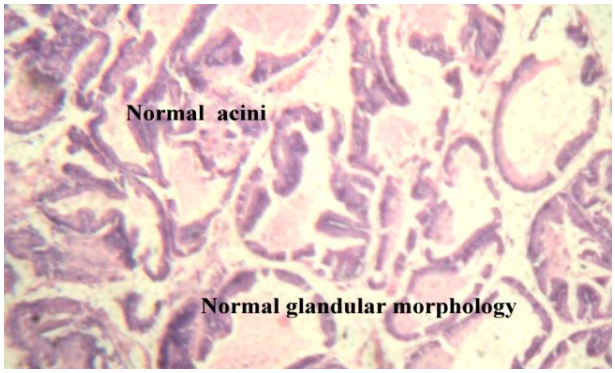

Fig.1.c: Group I

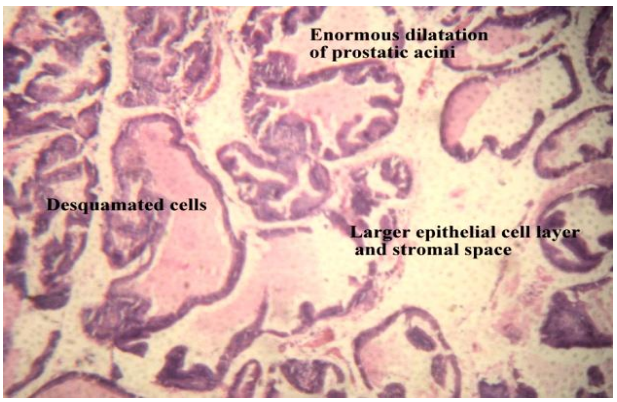

Fig.1.d: Group II

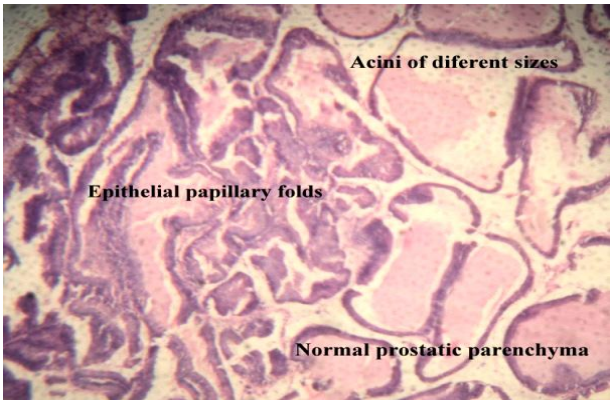

Fig.1.e: Group III

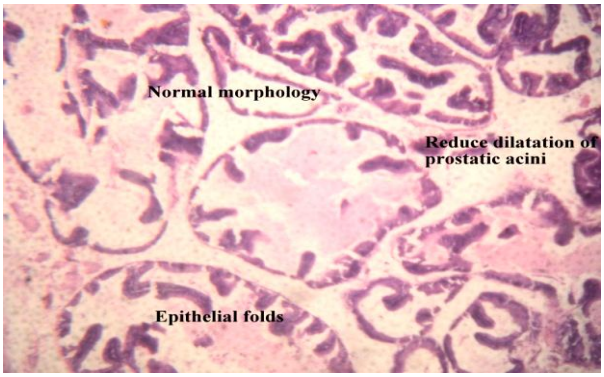

Fig.1.f: Group IV

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